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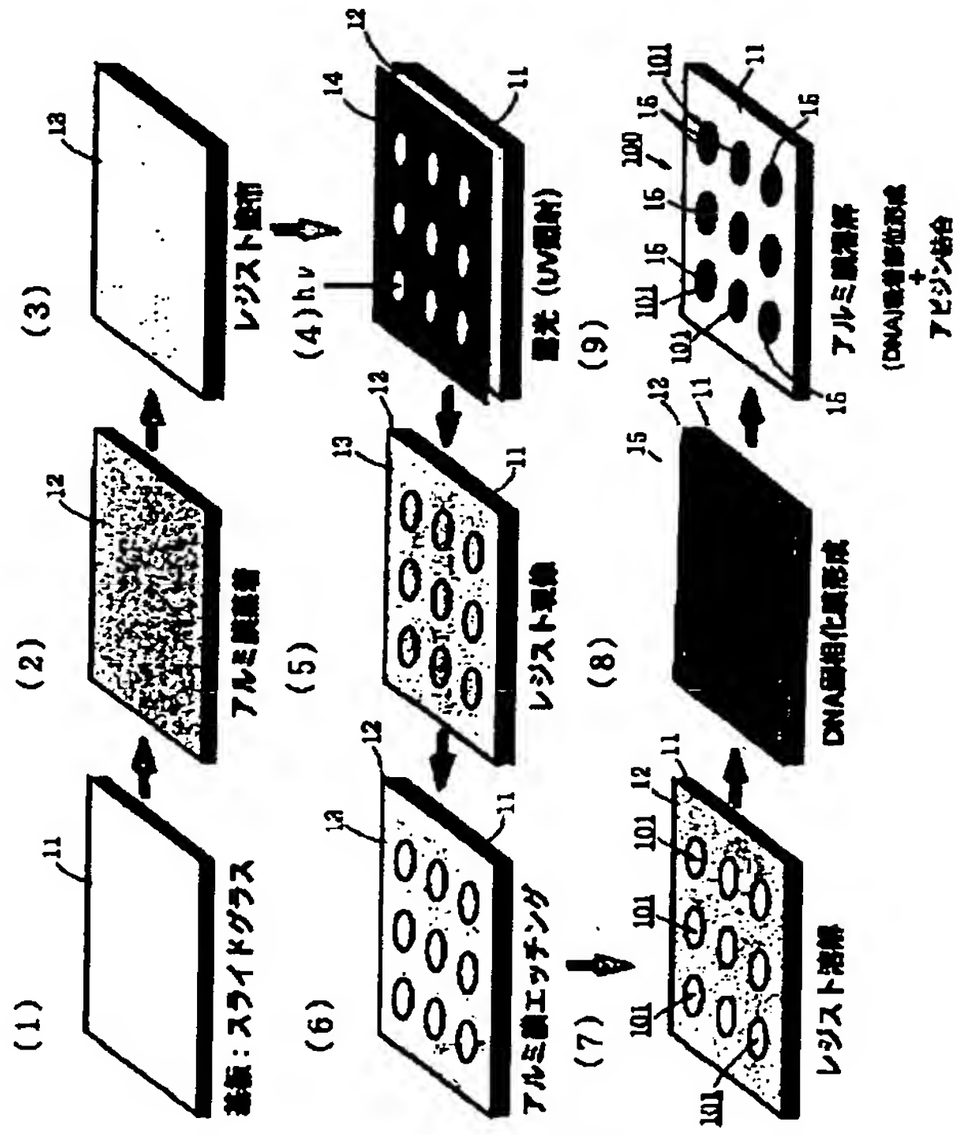
(71)出願人　000006792
理化学研究所
埼玉県和光市広沢２番１号
(71)出願人　390001421
学校法人早稲田大学
東京都新宿区戸塚町１丁目104番地
(72)発明者　田代　英夫
埼玉県和光市広沢２番１号　理化学研究所内
(74)代理人　100085660
弁理士　鈴木　均

最終頁に続く

(54)【発明の名称】　生体分子マイクロアレイ

(57)【要約】
【課題】　定量的な解析に使用できる、Ｓ／Ｎ比の高い生体分子マイクロアレイを提供する。
【解決手段】　フォトリソグラフィ技術およびエッチング技術を用いて、スライドグラス基板１１の表面のプローブ生体分子を付着させたい特定の部位１０１のみにアビジン分子が単層に固定された固相化膜１４を形成することにより表面処理基板１００を得る。特定の部位１０１の面積及び形状は全て均一であるため、各特定の部位１０１に固定されたビオチン分子の数もほぼ均一である。したがって各特定の部位１０１に結合するアビジン分子の数は同一になる。この表面処理基板１００の各特定の部位１０１に、ビオチン化処理したプローブＤＮＡを含む溶液をスポットすることにより、ＤＮＡマイクロアレイを得る。ＤＮＡマイクロアレイは、各特定の部位１０１に固定されているアビジン分子の数が同一であるので、各特定の部位１０１に結合するプローブＤＮＡ２１の数も同一である。



【特許請求の範囲】

【請求項１】　プローブ生体分子を含む溶液を基板表面にスポットすることにより、当該溶液中のプローブ生体分子が基板表面の特定の部位のみに受容され固定化されるように表面処理してなる基板であって、
前記基板表面のほぼ全面にわたり、前記プローブ生体分子を各々定量的に受容し得る複数の微細なプローブ生体分子受容固相部を規則的に設けたことを特徴とする生体分子マイクロアレイ用基板。

【請求項２】　前記プローブ生体分子受容固相部は、アビジン、ストレプトアビジン、ビオチン、アミノ基、カルボニル基、水酸基、スクシニイド基、マレイド基、チオール基のうちのいずれかの固相化剤からなることを特徴とする請求項１に記載の生体分子マイクロアレイ用基板。

【請求項３】　前記基板は、ガラス基板、シリコン基板、プラスチック基板、金基板、銀基板のうちのいずれかであることを特徴とする請求項１または２に記載の生体分子マイクロアレイ用基板。

【請求項４】　前記プローブ生体分子受容固相部は、基板表面に結合したビオチン分子の末端にアビジン分子が単層に結合したものであることを特徴とする請求項１～３のいずれかに記載の生体分子マイクロアレイ用基板。

【請求項５】　請求項１～４のいずれかに記載の基板の前記プローブ生体分子受容固相部にプローブ生体分子が結合していることを特徴とする生体分子マイクロアレイ。

【請求項６】　前記プローブ生体分子は、ＤＮＡ、ＲＮＡ、ＰＮＡまたはタンパク質であることを特徴とする請求項５に記載の生体分子マイクロアレイ。

【請求項７】　前記プローブ生体分子はビオチンを標識した生体分子であり、前記プローブ生体分子受容固相部にビオチンーアビジン結合により結合していることを特徴とする請求項５または６に記載の生体分子マイクロアレイ。

【請求項８】　請求項１～４のいずれかに記載の生体分子マイクロアレイ用基板を製造するための方法であって、
フォトリソグラフィ技術およびエッチング技術を用いて、特定の部位のみ前記プローブ生体分子受容固相部を設ける工程を含むことを特徴とする生体分子マイクロアレイ用基板の製造方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本願発明は、検出すべきターゲット生体分子に対して相補的な塩基配列を有する一本鎖の生体分子をプローブとし、当該プローブ生体分子と生体由来の試料核酸とのハイブリダイゼーションにより形成される二本鎖の有無を検出することによってターゲット生体分子を検出する生体分子検出技術に属し、特

に、プローブ生体分子を含む溶液を基板の表面にスポットすることにより当該基板上に生体分子検出スポット部を形成してなる生体分子マイクロアレイに関するものである。

【０００２】

【従来の技術】生体由来の試料中に存在する生体分子（ＤＮＡ、ＲＮＡなど）を検出するためのデバイスとしてＤＮＡマイクロアレイ（ＤＮＡチップともよばれる）がある。ＤＮＡマイクロアレイによれば、数百～数万回分の生体分子検出処理もしくは塩基配列決定処理を一括して並列的に行うことが可能である。ＤＮＡマイクロアレイは、数平方センチメートル～十数平方センチメートルのガラス基板やシリコン基板上に数百～数万のＤＮＡ検出ポイント（スポット部）を整然と配置してなる。それぞれのＤＮＡ検出ポイントには予め既知の塩基配列を持った一本鎖の核酸ポリマー（遺伝子断片）がプローブ（検出子）として一種類ずつ固定されている。つまり、ＤＮＡマイクロアレイ上にはたくさんの種類の核酸プローブが整列している。このＤＮＡマイクロアレイ上に、蛍光物質でラベリング（標識）した試料核酸の水溶液を流すと、試料核酸中の核酸ポリマーの塩基配列がプローブと相補的である場合のみ両者がハイブリダイズし、洗浄後も、ＤＮＡマイクロアレイ上にプローブとハイブリダイズしたターゲット核酸ポリマーだけが残存する。ＤＮＡマイクロアレイ上に残存したターゲット核酸ポリマー中の蛍光物質が発する蛍光を検出することにより、試料核酸中にターゲット核酸ポリマーが存在するか否かを判定できる。

【０００３】ＤＮＡマイクロアレイは、製造法によってフォトリソグラフィ型とスポッティング型の２種類に大別できる。フォトリソグラフィ型では、半導体集積回路の製造プロセスで用いられるフォトリソグラフィによって基板（あるいはシート）上で所望の多種類のＤＮＡ（オリゴヌクレオチド）を合成する製造方法がとられ、高密度のＤＮＡ検出ポイントを有するＤＮＡマイクロアレイが実用化されている（米国特許５７４４３０５、５４４５９３４等参照）。一方、スポッティング型では、固相化剤（ポリリジンまたはアミノシラン）をスライドガラスの全面にコーティングした基板（あるいはシート）を用い、その基板上に、あらかじめ調製したプローブＤＮＡを含む水滴を一つ一つスポットして載せた後、乾燥させることにより、ＤＮＡ検出スポットを形成する製造方法がとられる（米国特許５８７５２２等参照）。

【０００４】

【発明が解決しようとする課題】上述した２種類のＤＮＡマイクロアレイには、以下のような特性の違いがある。フォトリソグラフィ型のＤＮＡマイクロアレイは、ＤＮＡ検出ポイントを細かくでき、ＤＮＡを均一に生やすことができるため、高い測定感度とその再現性を保証できる点、ＳＮＰ（一塩基多型）分析に使用できる点で

優れている。しかしながら、マスクは１塩基合成するために１枚必要であり、塩基はＡ、Ｔ、Ｇ、Ｃと４種類あるので、少なくとも４枚のマスクが必要となる。たとえば２０塩基の長さのプローブを合成するには８０枚のマスクが必要である。マスクは１枚数十万円と高価であり、ＤＮＡマイクロアレイを作るためには数千万円の費用がかかる。このため、一部の研究機関でしか使用されていないのが現状である。

【０００５】スポッティング型のＤＮＡマイクロアレイは、プローブＤＮＡを含む水滴を基板上に載せて乾かす方法を用いるため、基板上に固定されるＤＮＡの密度と均一さが保証されない。すなわち、ＤＮＡ検出スポット部の寸法や形状が不均一になるため、各ＤＮＡ検出スポット部に固定されているＤＮＡ量にばらつきが生じる。このためスポッティング型のＤＮＡマイクロアレイは、定性的な解析には使用できても、定量的な解析には向いていなかった。すなわち、ターゲット生体分子とのハイブリダイゼーションが生じたＤＮＡ検出スポット部の有無は検出できても、各ＤＮＡ検出スポット部でハイブリダイゼーションしたターゲット生体分子の量を測定することはできなかった。また、ＤＮＡ検出スポット部の周囲に付着した固相化剤の存在により、ターゲットＤＮＡが非特異的に基板上に吸着し、ノイズの上昇を引き起こし、Ｓ／Ｎ比を低下させていた。本願発明は、このような事情の下に創案されたものであり、その目的は、定量的な解析に使用でき且つＳ／Ｎ比の高いスポッティング型の生体分子マイクロアレイを提供することにある。

【０００６】

【課題を解決するための手段】上記目的を達成するために、本願発明では以下の手段を採用する。本発明に係る生体分子マイクロアレイ用基板は、基板表面のほぼ全面にわたり、前記プローブ生体分子を各々定量的に受容し得る複数の微細なプローブ生体分子受容固相部を規則的に設けたことを特徴とする。本発明の生体分子マイクロアレイ用基板において、前記プローブ生体分子受容固相部は、アビジン、ストレプトアビジン、ビオチン、アミノ基、カルボニル基、水酸基、スクシニイド基、マレイド基、チオール基のうちのいずれかの固相化剤からなる。また、前記基板は、ガラス基板、シリコン基板、プラスチック基板、金基板、銀基板のうちのいずれかである。また、前記プローブ生体分子受容固相部は、基板表面に結合したビオチン分子の末端にアビジン分子が単層に結合したものである。また、前記特定の部位の径は５０～２００ミクロン、前記特定の部位同士の間隔は１００～５００ミクロンであることが望ましい。ここで、前記特定の部位の径とは、当該特定の部位の形状が円形の場合は直径、正方形の場合は一片の長さを意味する。また、前記特定の部位の形状が、前記生体分子マイクロアレイの生体分子検出スポット部の撮像に使用する固体撮像素子の画素の形状と略一致していることが望ましい。

本発明に係る生体分子マイクロアレイは、請求項１～４のいずれかに記載の基板の前記プローブ生体分子受容固相部にプローブ生体分子を結合させたものであることを特徴とする。本発明の生体分子マイクロアレイにおいて、前記プローブ生体分子は、ＤＮＡ、ＲＮＡ、ＰＮＡまたはタンパク質である。また、前記プローブ生体分子はビオチンを標識した生体分子であり、前記プローブ生体分子受容固相部にビオチンーアビジン結合により結合している。本発明に係る製造方法は、前記生体分子マイクロアレイ用基板を製造する方法であって、フォトリソグラフィ技術およびエッチング技術を用いて、特定の部位のみ前記プローブ生体分子受容固相部を設ける工程を含むことを特徴とする。

【０００７】

【発明の実施の形態】以下、本発明の実施の形態について図面を参照して詳細に説明する。まず、本発明に係るＤＮＡマイクロアレイ用基板（以下、単に表面処理基板と記す。）について説明する。図１は本発明に係る表面処理基板の製造方法の一例を示す製造工程図である。図中、１００は本発明に係る表面処理基板であり、この表面処理基板１００は、プローブＤＮＡが特定の部位１０１のみに付着するように表面処理してなる。表面処理基板１００の製造工程は以下のとおりである。

（１）基板洗浄工程：スライドグラス基板１１を洗浄し不純物を取り除く。

（２）アルミニウム膜蒸着工程：スライドグラス基板１１の表面に、アルミニウム膜１２を蒸着（コーティング）する。

（３）フォトレジストの塗布工程：アルミニウム膜１２の表面にネガ型のフォトレジストを塗布（コーティング）する。

（４）露光工程：フォトマスク１４を通して（３）の基板上の特定の部位１０１にのみ光（ｈν）を照射する。

（５）現像工程：（４）の基板上のフォトレジスト１３を現像する。この段階で特定の部位１０１のフォトレジスト１３が除去される。

（６）エッチング工程：（５）の基板上のアルミニウム膜をエッチングする。この段階で、特定の部位１０１のアルミニウム膜１２が除去される。

（７）レジスト除去工程：（６）の基板上のフォトレジスト１３をアセトンにより溶解し除去する。この段階で、スライドグラス基板１１の表面が特定の部位１０１のみ露出する。

（８）ＤＮＡ固相化膜形成工程：（７）の基板上に、プローブＤＮＡを吸着し固相化する固相化剤を塗布し、ＤＮＡ固相化膜１５を形成する。この工程は、具体的には、アミノシラン処理による基板表面へのアミノ基導入工程と、ビオチンスクシニイドによる基板表面のアミノ基へのビオチン導入工程とからなる。

（９）ＤＮＡ付着部位形成工程：（８）の基板上のアル

ミニウム膜１２を酸、アルカリまたはキレート剤により溶解させて除去する。この段階で、スライドグラス基板１１の表面の特定の部位１０１にのみＤＮＡ固相化膜１５が形成される。

（１０）アビジン結合工程：（９）の基板上にアビジン溶液を導入し、特定の部位１０１に形成されたＤＮＡ固相化膜１５のビオチン分子の末端にアビジン分子を単層に結合させる。

以上の（１）～（１０）の工程を経ることにより、スライドグラス基板１１の表面の特定の部位１０１のみにアビジンが単層に固定されたＤＮＡマイクロアレイ基板１００が得られる。特定の部位１０１の直径は２００ミクロン以下、特定の部位１０１同士の間隔は４００ミクロン以下である。

【０００８】図２に各ＤＮＡ固相化膜１５のビオチン分子の末端にアビジン分子が単層に結合する過程を示す。各基板の表面に形成される特定の部位１０１の面積及び形状は全て均一であるため、各特定の部位１０１に固定されたビオチン分子２３の数もほぼ均一である。したがって、各特定の部位１０１に結合するアビジン分子の数は均等になる。すなわち、各特定の部位１０１に固定されたビオチンの数に多少ばらつきがあっても、アビジン分子の方がビオチン分子よりも遙かに大きいため、各特定の部位１０１に固定されるアビジン分子の数は一定になる。特定の部位１０１の形状や寸法、特定の部位１０１同士の間隔は、露光工程で使用するフォトマスクを変えることによって任意に変更できる。したがって、特定の部位１０１に固定するアビジン分子の数も、フォトマスクを変えることによって任意に制御できる。

【０００９】次に、本発明に係るＤＮＡマイクロアレイについて説明する。本発明に係るＤＮＡマイクロアレイは、図１の方法で製造された表面処理基板１００の各特定の部位１０１に、各々塩基配列の異なるプローブＤＮＡを含む溶液をスポットすることにより製造される。図３に本発明に係るＤＮＡマイクロアレイの製造方法の一例を示す。図中、１１０はプローブＤＮＡ２１を含む溶液１１１をＤＮＡマイクロアレイ基板１００上にスポットするためのアレイヤである。プローブＤＮＡ２１には、予めビオチンを標識したＤＮＡ（ビオチン化ＤＮＡ）を使用する。アレイヤ１１０は、毛細管作用により溶液１１１を一定量保持できるようになっており、溶液１１１を保持したアレイヤ１１０の先端を表面処理基板１００上の特定の部位１０１に突き当てることにより、一定量の溶液１１１が特定の部位１０１に供給される。その結果、溶液１１１中のプローブＤＮＡ２１がＤＮＡマイクロアレイ基板１００上の特定の部位１０１に固定されている各アビジン分子２２に１つずつ結合する（ビオチン化ＤＮＡの固相化）。各特定の部位１０１に固定されているアビジン分子２２の数は同一であるので、各特定の部位１０１に結合するプローブＤＮＡ２１の数も

同一である（図４参照）。各特定の部位１０１に固定するアビジン分子の数は、上述したように、露光工程で使用するフォトマスクを変えて特定の部位１０１の形状や寸法を変更することにより任意に変更できる。したがって、各特定の部位１０１に固定するプローブＤＮＡ２１の数も、露光工程で使用するフォトマスクの変更により任意に制御でき、各特定の部位１０１にスポットする溶液１１１のプローブＤＮＡ濃度にばらつきがあっても、常に一定数のプローブＤＮＡ２１を各特定の部位１０１に固定することができる。

【００１０】図５に表面処理基板上の特定の部位にスポットする溶液中のＤＮＡ量（濃度）と特定の部位に固定化されるＤＮＡ量との関係の測定例を示す。この測定結果から、3×10^{9}～5×10^{9}個のプローブＤＮＡをスポットすれば特定の部位に固定化されるプローブＤＮＡ量が一定になることがわかる。ＤＮＡマイクロアレイ上のＤＮＡ検出スポット部は、全て同一形状、同一面積であり、しかも全検出スポット部に同数ずつプローブＤＮＡ２１が固定されているので、このＤＮＡマイクロアレイによれば定量的な解析が可能になる。ＤＮＡマイクロアレイ上におけるＤＮＡ吸着部位が特定の部位１０１すなわちＤＮＡ検出スポット部のみに制限されるので、ＤＮＡ検出スポット部の周囲にＤＮＡが非特異的は吸着を起こすのを防止できる。したがって、蛍光検出時のノイズ（不要光）の減少により、Ｓ／Ｎ比を向上させることができる。さらに、ＤＮＡ検出スポット部の形状すなわち前記特定の部位の形状を、撮像に使用する固体撮像素子（ＣＣＤセンサ、ＣＭＯＳセンサなど）の画素の形状と一致させておくことにより、Ｓ／Ｎ比をより向上させることができる。

【００１１】なお、本発明は以上の実施の形態に限定されるものではない。たとえば、本発明に係る表面処理基板の製造方法は上記の実施の形態に限定されない。すなわち、図１に示した製造方法では、基板１１の表面に、先ずアルミニウム膜１２、ポジ型のフォトレジスト１３を順次積層する。次に特定の部位１０１を規則的に配列してあるフォトマスクを通して、フォトレジストの特定の部位のみ露光し、現像液に浸すことで、特定の部位１０１のみフォトレジストが溶解し、特定の部位１０１のみアルミニウム膜１２が露出する。その後、アルミニウム膜１２を酸性のエッチング溶液によりエッチングすることにより、特定の部位１０１のアルミニウム膜１２が溶解され、基板１１の特定の部位１０１のガラス表面が露出する。その上にＤＮＡ固相化膜１５を塗布し、アルミニウム膜１２を溶解することにより、基板１１の表面の特定の部位１０１にのみＤＮＡ固相化膜１５を残すことができる。別な方法として、基板１１の表面全体に最初からＤＮＡ固相化膜１５を形成し、特定の部位１０１のみＤＮＡ固相化膜１５を露出させる方法を採用してもよい。この場合には、基板１１の表面全体にＤＮＡ固相

化膜１５およびアルミニウム膜１２を順次積層形成した後、アルミニウム膜１２上にポジ型のフォトレジスト１３を積層形成し、フォトマスクを通して特定の部位１０１にのみ露光し、上記と同じように、現像とエッチングとを行うことにより、特定の部位１０１のみＤＮＡ固相化膜１５を露出させることができる。また、フォトレジストはポジ型である必要はなく、ネガ型のフォトレジストも使用可能であることは無論である。

【００１２】また、上記実施の形態では、ＤＮＡマイクロアレイ、すなわちプローブ生体分子としてＤＮＡを固定した生体分子マイクロアレイについて説明したが、プローブ生体分子としてＲＮＡ、ＰＮＡ、蛋白質などを用いたものも本発明の生体分子マイクロアレイに含まれる。また、表面処理基板に用いる基板は、スライドグラス基板に限るものではなく、透明ガラス基板、シリコン基板、プラスチック基板、金基板、銀基板などでもよい。また、固相化剤に付いても、アビジンを固定（露出）したものに限らず、前記特定の部位に固定化したいプローブ生体分子との結合性を考慮して、固定化するプローブ生体分子数の定量化に最も適切と思われる物質を使用すればよい。

【００１３】

【発明の効果】以上説明したように、本発明は以下のような優れた効果を奏する。本発明に係る生体分子マイクロアレイ用基板は、プローブ生体分子が基板表面の特定の部位のみに付着するように表面処理されているので、プローブ生体分子を含む溶液を基板表面にスポットすることにより、生体分子検出スポット部の外の領域にプローブ生体分子が付着するのを防止して、Ｓ／Ｎ比の高い生体分子マイクロアレイを得ることができる。また、前記特定の部位の面積及び形状を変えることにより、生体分子検出スポット部に固定化するプローブ生体分子の量を制御することができる。前記特定の部位の面積及び形状を全て同一とすれば、全生体分子検出スポット部のプローブ生体分子の量を一定にすることができ、本発明に係る生体分子マイクロアレイが得られる。本発明に係る生体分子マイクロアレイによれば、各生体分子検出スポット部に固定化されているプローブ生体分子の量が一定であるので、ターゲット生体分子の定量的な解析に使用できる。また、プローブ生体分子を特定の部位に固定化することで生体分子検出スポット部が形成されているので、生体分子検出スポット部の外の領域におけるターゲット生体分子の非特異的吸着を防止し、Ｓ／Ｎ比の高い測定を行うことができる。本発明に係る製造方法によれば、プローブ生体分子を吸着し固相化する固相化膜をフォトリソグラフィなどの精密加工技術を用いて基板表面の特定の部位のみに形成することにより、本発明に係る生体分子マイクロアレイ用表面処理基板を高精度に製造することができる。

【図面の簡単な説明】

【図１】本発明に係る表面処理基板の製造方法の一例を示す製造工程図である。

【図２】各ＤＮＡ固相化膜のビオチン分子の末端にアビジン分子が結合する過程に関する説明図である。

【図３】本発明に係るＤＮＡマイクロアレイの製造方法の一例を示す説明図である。

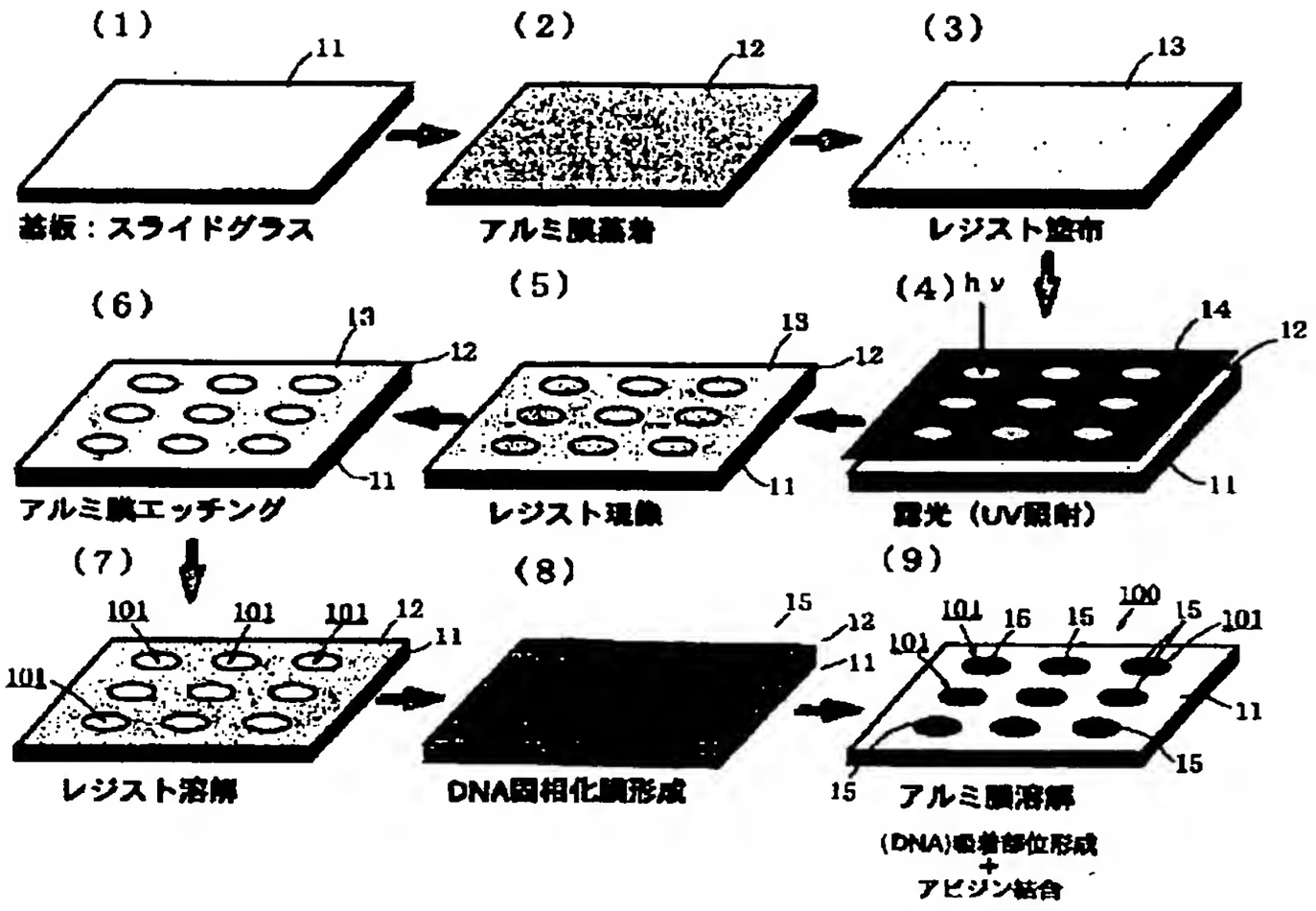
【図４】特定の部位に固定されているアビジン分子にプローブＤＮＡ（ビオチン化ＤＮＡ）が結合する過程に関する説明図である。

【図５】表面処理基板上の特定の部位にスポットする溶液中のＤＮＡ量（濃度）と特定の部位に固定化されるＤＮＡ量との関係の測定結果を示す図である。

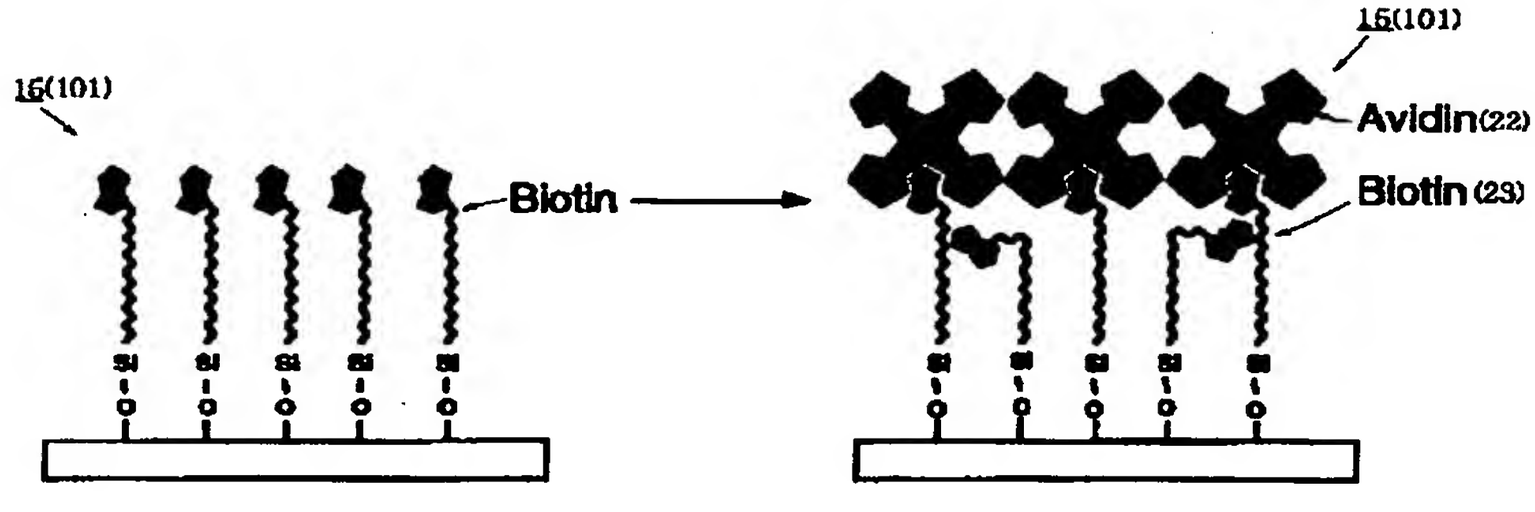
【符号の説明】

１１：スライドグラス基板
１２：アルミニウム膜
１３：フォトレジスト
１４：フォトマスク
１５：固相化膜
２１：プローブＤＮＡ
２２：アビジン分子
２３：ビオチン分子
１００：ＤＮＡマイクロアレイ表面処理基板
１０１：特定の部位
１１０：アレイヤ
１１１：溶液

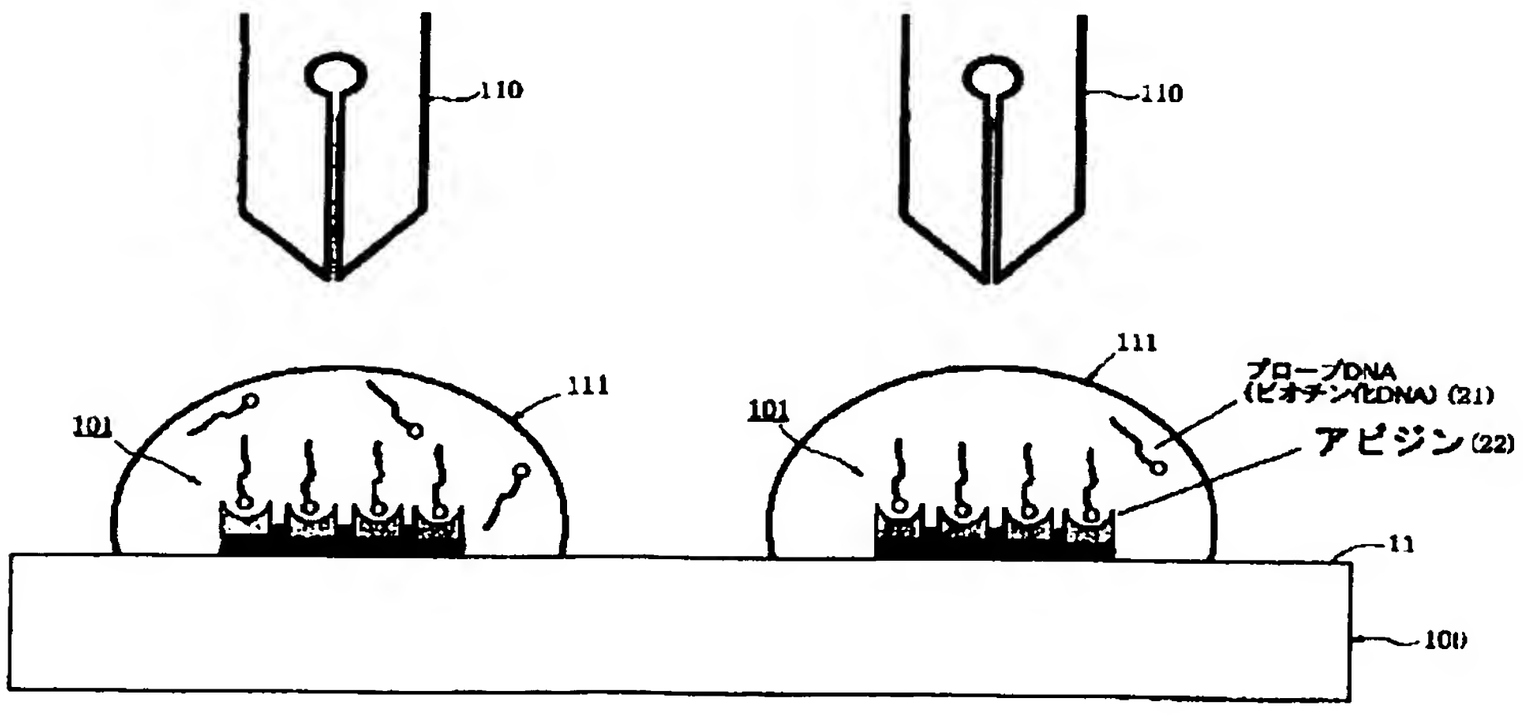
【図１】


(1)
11
基板：スライドグラス
(2)
12
アルミ膜蒸着
(3)
13
レジスト塗布
(4) hν
14
12
11
露光（UV照射）
(5)
13
12
11
レジスト現像
(6)
13
12
11
アルミ膜エッチング
(7)
101
101
101
12
11
101
レジスト溶解
(8)
15
12
11
DNA固相化膜形成
(9)
101
16
15
100
15
101
11
15
15
アルミ膜溶解
(DNA)吸着部位形成
+
アビジン結合

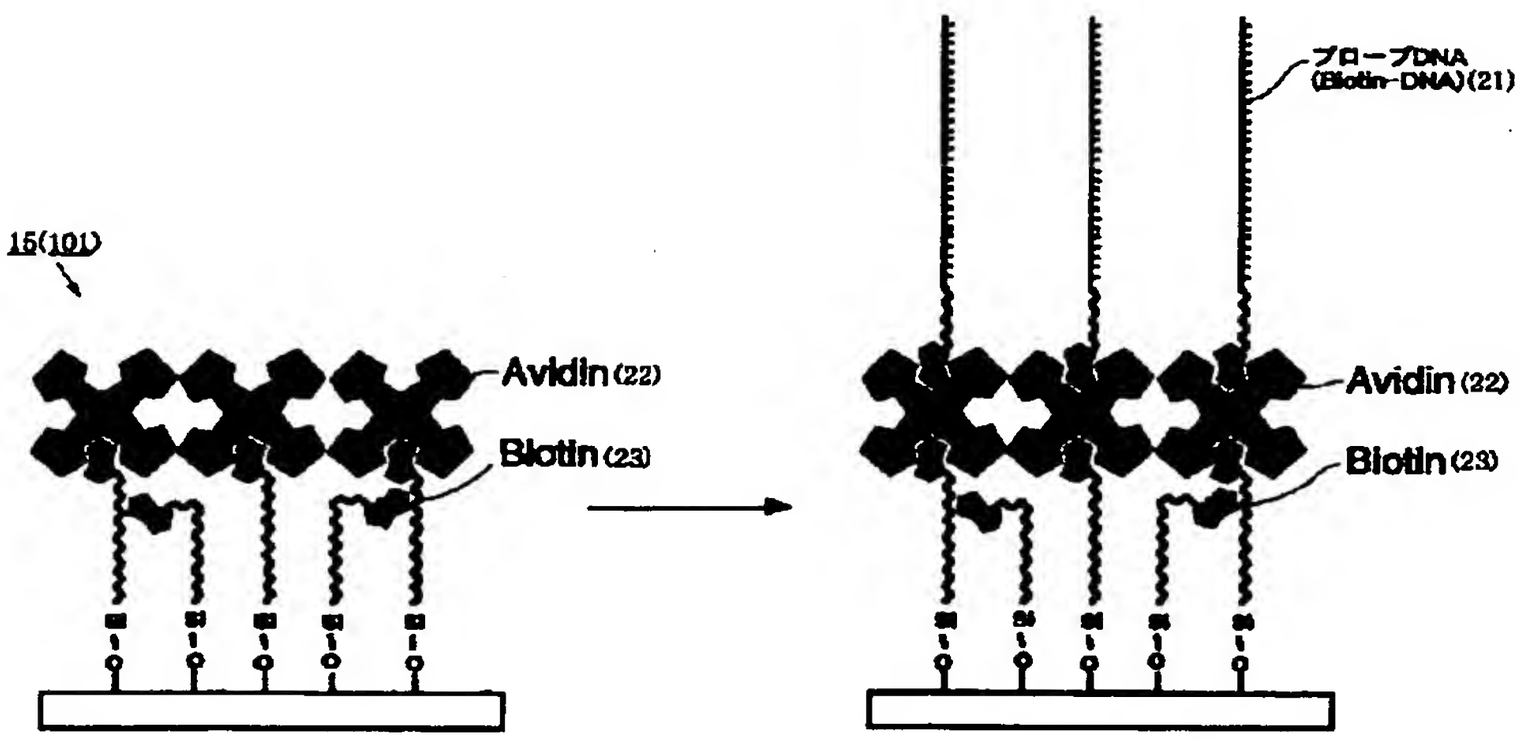
【図２】


15(101)
Biotin
15(101)
Avidin(22)
Biotin(23)

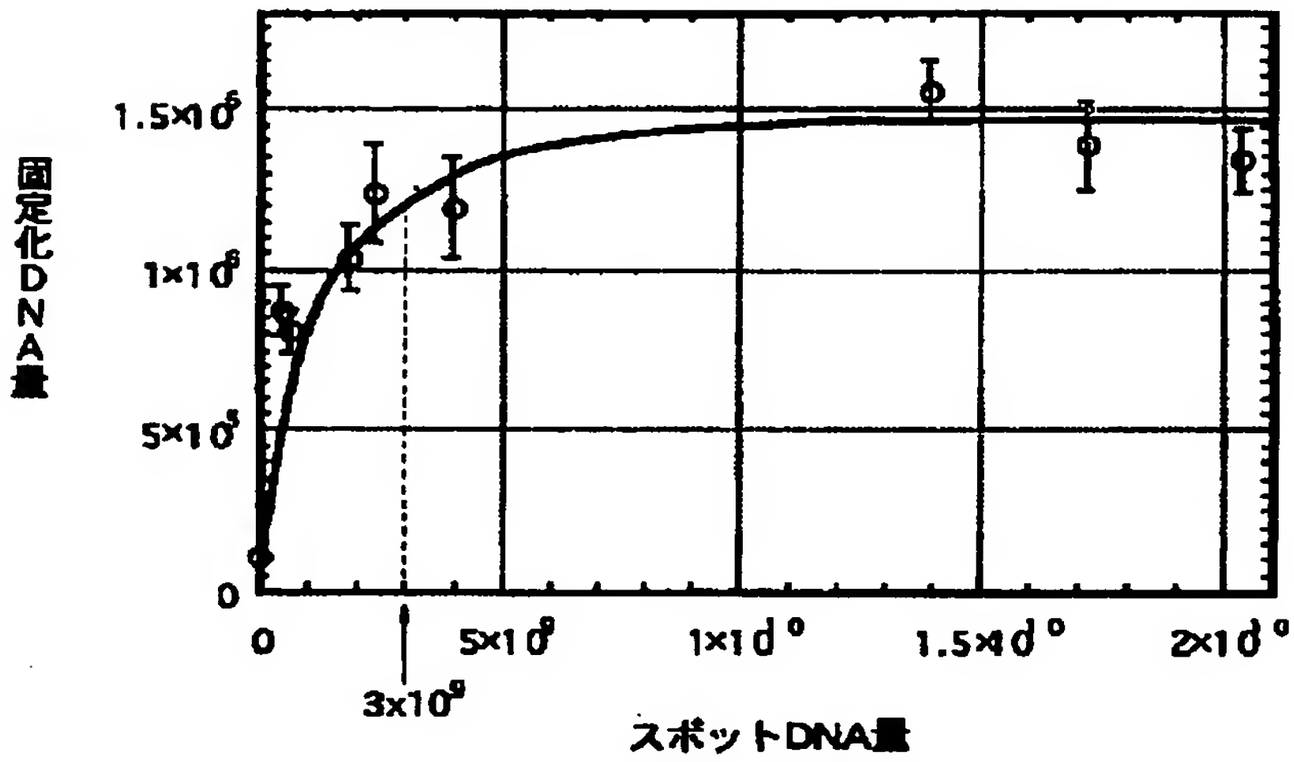
【図３】


110
110
111
101
111
101
プローブDNA
(ビオチン化DNA) (21)
アビジン(22)
11
100

【図４】



【図５】



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(72)発明者　近藤　恭光
埼玉県和光市広沢２番１号　理化学研究所内
(72)発明者　橘内　徳司
埼玉県和光市広沢２番１号　理化学研究所内
(72)発明者　竹中　繁織
福岡県古賀市舞の里４丁目23－21
(72)発明者　松本　和子
東京都新宿区戸塚町１丁目104番地　学校法人早稲田大学内
(72)発明者　野島　高彦
東京都新宿区戸塚町１丁目104番地　学校法人早稲田大学内

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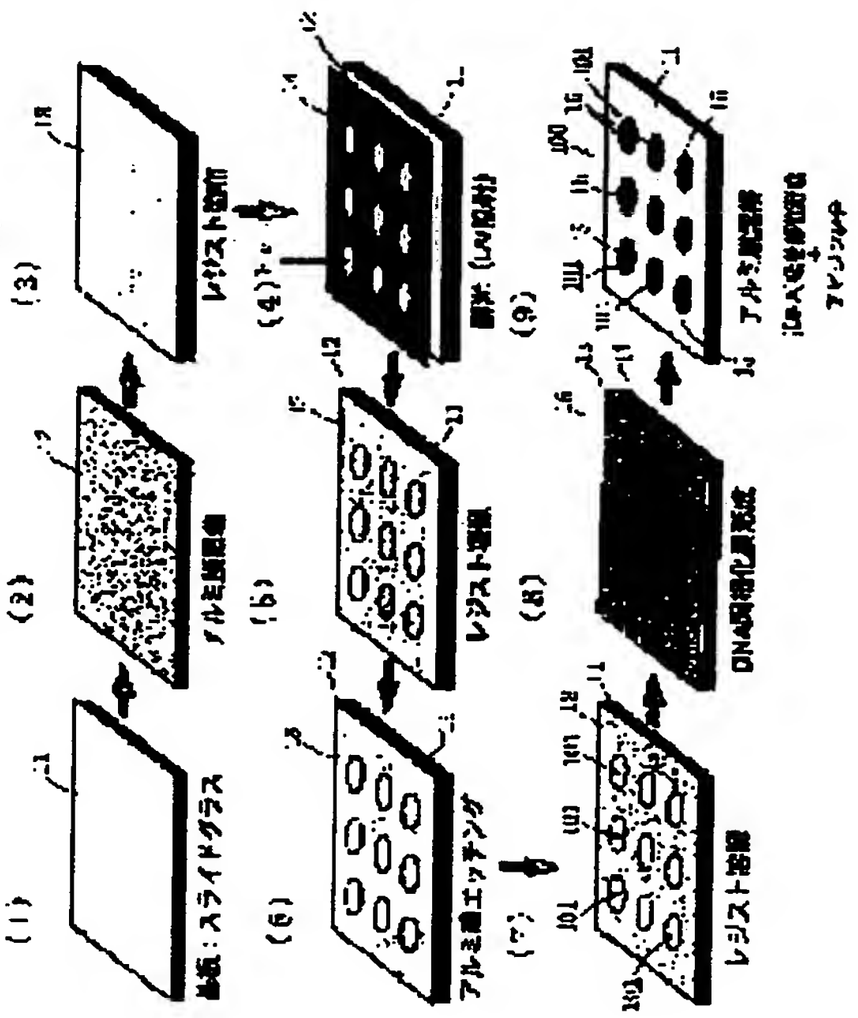
(71)Applicant : INST OF PHYSICAL & CHEMICAL RES
UNIV WASEDA
(72)Inventor : TASHIRO HIDEO
KONDO YASUMITSU
KITSUNAI TOKUJI
TAKENAKA SHIGEORI
MATSUMOTO KAZUKO
NOJIMA TAKAHIKO

(54) BIOMOLECULE MICROARRAY

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a biomolecule microarray which can be used for quantitative analyses and has a high S/N ratio.
SOLUTION: A photolithography technique and an etching technique are used to form an immobilizing film 14 in which avidin molecules are immobilized in a single layer at only specific probe biomolecule adhesion-desired sites 101 on the surface of a slide glass substrate 11, thus obtaining the surface-treated substrate 100. Since the areas and shapes of the specific sites all are uniform, the numbers of the biotin molecules immobilized on the specific sites 101 are also approximately uniform. Thereby, the numbers of the avidin molecoles bound to the specific sites 101 are identical. The specific sites 101 of the surface- treated substrate 100 are spotted with a solution containing probe DNA treated with biotin to obtain the DNA microarray. Since the numbers of the avidin molecules immobilized on the specific sites 101 are identical in the DNA microarrays, the number of probe DNAs 21 bound to the specific sites 101 is also identical.



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[Date of extinction of right]

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CLAIMS

[Claim(s)]
[Claim 1] The substrate for biomolecule microarrays which is a substrate which carries out surface preparation and becomes as the probe biomolecule in the solution concerned is received and fixed by only the specific part on the front face of a substrate by carrying out the spot of the solution containing probe biomolecule to a substrate front face, and is characterized by to prepare regularly two or more detailed probe biomolecule acceptance solid phase sections on said front face of a substrate which migrate to the whole surface mostly and may receive said probe biomolecule quantitatively respectively.
[Claim 2] Said probe biomolecule acceptance solid phase section is a substrate for biomolecule microarrays according to claim 1 characterized by consisting of a solid phase-ized agent of the avidin, streptoavidin, a biotin, the amino group, a carbonyl group, a hydroxyl group, a SUKUSHI need radical, a MAREIDO radical, or the thiol groups.
[Claim 3] Said substrate is a substrate for biomolecule microarrays according to claim 1 or 2 characterized by being a glass substrate, a silicon substrate, a plastic plate, a golden substrate, or the silver substrates.
[Claim 4] Said probe biomolecule acceptance solid phase section is a substrate for biomolecule microarrays according to claim 1 to 3 characterized by an avidin molecule combining with a monolayer at the end of the biotin molecule combined with the substrate front face.
[Claim 5] The biomolecule microarray characterized by probe biomolecule having combined with said probe biomolecule acceptance solid phase section of a substrate according to claim 1 to 4.
[Claim 6] Said probe biomolecule is a biomolecule microarray according to claim 5 characterized by being DNA, RNA, PNA, or protein.
[Claim 7] Said probe biomolecule is a biomolecule microarray according to claim 5 or 6 which is the biomolecule which carried out the indicator of the biotin, and is characterized by having combined with said probe biomolecule acceptance solid phase section by biotin-avidin association.
[Claim 8] The manufacture approach of the substrate for biomolecule microarrays which is an approach for manufacturing the substrate for biomolecule microarrays according to claim 1 to 4, and is characterized by including the process in which only a specific part prepares said probe biomolecule acceptance solid phase section using a photolithography technique and an etching technique.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] The invention in this application uses as a probe biomolecule of the single strand which has a complementary base sequence to the target biomolecule which should be detected, belongs to the biomolecule detection technique detect target biomolecule by detecting the existence of the double strand formed of the hybridization of the probe biomolecule concerned and the sample nucleic acid of the living body origin, and relates to the biomolecule microarray which comes to form the biomolecule detection spot section on the substrate concerned by carrying out the spot of the solution which contains probe biomolecule especially on the surface of a substrate.
[0002]
[Description of the Prior Art] There is a DNA microarray (called a DNA chip) as a device for detecting the biomolecules (DNA, RNA, etc.) which exist in the sample of the living body origin. According to the DNA microarray, it is possible for biomolecule detection processing or base-sequence-determination processing of hundreds - tens of thousands batches to be put in block, and to perform it in juxtaposition. A DNA microarray comes to arrange the DNA detection point (spot section) of hundreds - a-10,000 number on a several square centimeters - about ten square centimeters glass substrate or a silicon substrate tidily. One kind of nucleic-acid polymer (gene fragment) of a single strand which had a known base sequence beforehand is being fixed to each DNA detection point at a time as a probe (sensor). That is, on a DNA microarray, many kinds of nucleic acid probes have aligned. If the water solution of the sample nucleic acid which carried out labeling (indicator) with the fluorescent material on this DNA microarray is poured, only when the base sequence of the nucleic-acid polymer in a sample nucleic acid is as complementary as FUROBU, both will hybridize, and also even in after washing, only a probe and the target nucleic-acid polymer to which it hybridized remain on a DNA microarray. By detecting the fluorescence which the fluorescent material in the target nucleic-acid polymer which remained on the DNA microarray emits, it can judge whether a target nucleic-acid polymer exists in a sample nucleic acid.
[0003] A DNA microarray can be divided roughly into two kinds, a photolithography mold and a spotting mold, according to a manufacturing method. In the photolithography mold, the manufacture approach which compounds DNA (oligonucleotide) of desired varieties on a substrate (or sheet) by the photolithography used in the manufacture process of a semiconductor integrated circuit is taken, and the DNA microarray which has the DNA detection point of high density is put in practical use (refer to U.S. Pat. No. 5744305 and 5445934 grade). On the other hand, in a spotting mold, after carrying out the spots of the waterdrop containing the probe DNA which prepared beforehand the solid phase-ized agent (the poly lysine or amino silane) on the substrate using the substrate (or sheet) with which the whole surface of slide glass was coated one by one and carrying it, the manufacture approach which forms a DNA detection spot is taken by making it dry (reference, such as U.S. Pat. No. 587522).
[0004]
[Problem(s) to be Solved by the Invention] There is a difference among the following properties

in two kinds of DNA microarrays mentioned above. Since the DNA microarray of a photolithography mold can make the DNA detection point fine and can grow DNA to homogeneity, it is excellent in the point that high sensitometry and its repeatability can be guaranteed, and the point which can be used for SNP (a little salt radical polymorphism) analysis. However, one mask is required in order to compound one base, and since there are four kinds of bases with A, T, G, and C, at least four masks are needed. For example, 80 masks are required to compound the probe of the die length of 20 bases. The mask is as expensive as hundreds of thousands of yen per sheet, and in order to make a DNA microarray, it requires tens of millions of yen costs. For this reason, the present condition is used only by some research facilities.
[0005] In order to use for the DNA microarray of a spotting mold the approach of carrying the waterdrop containing probe DNA on a substrate, and drying it, the consistency and uniformity of DNA which are fixed on a substrate are not guaranteed. That is, since the dimension and configuration of the DNA detection spot section become an ununiformity, dispersion arises in the amount of DNA currently fixed to each DNA detection spot section. For this reason, even if the DNA microarray of a spotting mold was applicable to qualitative analysis, it was not fit for quantitive analysis. That is, even if the existence of the DNA detection spot section which the hybridization with target biomolecule produced was detectable, it was not able to measure the amount of the target biomolecule which carried out hybridization in each DNA detection spot section. Moreover, by existence of the solid phase-ized agent adhering to the perimeter of the DNA detection spot section, Target DNA adsorbed on the substrate nonspecific, the rise of a noise was caused, and the S/N ratio was reduced. It is originated under such a situation and the invention in this application has the purpose in being able to use it for quantitive analysis and offering the biomolecule microarray of the high spotting mold of a S/N ratio.
[0006]
[Means for Solving the Problem] In order to attain the above-mentioned purpose, the following means are adopted in the invention in this application. The substrate for biomolecule microarrays concerning this invention is characterized by preparing regularly the detailed probe biomolecule acceptance solid phase section of the plurality on the front face of a substrate which migrates to the whole surface mostly and may receive said probe biomolecule quantitatively respectively. In the substrate for biomolecule microarrays of this invention, said probe biomolecule acceptance solid phase section consists of a solid phase-ized agent of the avidin, streptoavidin, a biotin, the amino group, a carbonyl group, a hydroxyl group, a SUKUSHI need radical, a MAREIDO radical, or the thiol groups. Moreover, said substrates are a glass substrate, a silicon substrate, a plastic plate, a golden substrate, or the silver substrates. Moreover, an avidin molecule combines said probe biomolecule acceptance solid phase section with the end of the biotin molecule combined with the substrate front face at a monolayer. Moreover, as for spacing of 50-200 microns and said specific parts, it is [the path of said specific part] desirable that it is 100-500 microns. Here, as for the case of a diameter and a square, the path of said specific part means the die length of a piece, when the configuration of the specific part concerned is circular. Moreover, it is desirable for the configuration of said specific part to carry out abbreviation coincidence with the configuration of the pixel of the solid state image sensor used for the image pick-up of the biomolecule detection spot section of said biomolecule microarray. The biomolecule microarray concerning this invention is characterized by combining probe biomolecule with said probe biomolecule acceptance solid phase section of a substrate according to claim 1 to 4. In the biomolecule microarray of this invention, said probe biomolecule is DNA, RNA, PNA, or protein. Moreover, said probe biomolecule is the biomolecule which carried out the indicator of the biotin, and is combined with said probe biomolecule acceptance solid phase section by biotin-avidin association. The manufacture approach concerning this invention is an approach of manufacturing said substrate for biomolecule microarrays, and is characterized by including the process in which only a specific part prepares said probe biomolecule acceptance solid phase section using a photolithography technique and an etching technique.
[0007]
[Embodiment of the Invention] Hereafter, the gestalt of operation of this invention is explained to a detail with reference to a drawing. First, the substrate for DNA microarrays concerning this

invention (it is only hereafter described as a surface treatment substrate.) is explained. Drawing 1 is the production process Fig. showing an example of the manufacture approach of the surface treatment substrate concerning this invention. Among drawing, 100 are a surface-preparation substrate concerning this invention, surface preparation is carried out and this surface-preparation substrate 100 becomes so that probe DNA may adhere only to the specific part 101.
The production process of the surface treatment substrate 100 is as follows.
(1) Substrate washing process : wash the slide glass substrate 11 and remove an impurity.
(2) Aluminum film vacuum evaporationo process : vapor-deposit the aluminum film 12 on the front face of the slide glass substrate 11 (coating).
(3) The spreading process of a photoresist : apply the photoresist of a negative mold to the front face of the aluminum film 12 (coating).
(4) Exposure process : irradiate light (hnu) only to the specific part 101 on the substrate of (3) through a photo mask 14.
(5) Develop the photoresist 13 on the substrate of development process: (4). The photoresist 13 of the specific part 101 is removed in this phase.
(6) Etch the aluminum film on the substrate of etching process: (5). In this phase, the aluminum film 12 of the specific part 101 is removed.
(7) Dissolve with an acetone and remove the photoresist 13 on the substrate of resist removal process: (6). In this phase, the front face of the slide glass substrate 11 exposes only the specific part 101.
(8) On the substrate of DNA solid phase-ized film formation process: (7), apply the solid phase-ized agent which adsorbs probe DNA and solid-phase-izes it, and form the DNA solid phase-ized film 15. Specifically, this process consists of an amino-group installation process on the front face of a substrate by amino silanizing, and a biotin installation process to the amino group on the front face of a substrate by the biotin SUKUSHI need.
(9) Make it dissolve by the acid, alkali, or the chelating agent, and remove the aluminum film 12 on the substrate of DNA attachment site formation process: (8). The DNA solid phase-ized film 15 is formed only in the specific part 101 of the front face of the slide glass substrate 11 in this phase.
(10) Introduce an avidin solution on the substrate of avidin joint process: (9), and make a monolayer combine an avidin molecule with the end of the biotin molecule of the DNA solid phase-ized film 15 formed in the specific part 101.
By passing through the process of the above (1) - (10), the DNA microarray substrate 100 with which avidin was fixed only to the specific part 101 of the front face of the slide glass substrate 11 by the monolayer is obtained. Spacing of 200 microns or less and part 101 specific comrades of the diameter of the specific part 101 is 400 microns or less.
[0008] The process which an avidin molecule combines with a monolayer is shown in the end of the biotin molecule of each DNA solid phase-ized film 15 at drawing 2 . Since all of a specific area and the specific configuration of a part 101 which are formed in the front face of each substrate are homogeneity, its number of the biotin molecules 23 fixed to each specific part 101 is also almost uniform. Therefore, the number of the avidin molecules combined with each specific part 101 becomes equal. That is, even if some dispersion is in the number of the biotins fixed to each specific part 101, the number of the avidin molecules with which the direction of an avidin molecule is fixed to each specific part 101 since it is larger than a biotin molecule for whether your being Haruka becomes fixed. Spacing of the configuration of the specific part 101, a dimension, and part 101 specific comrades can be changed into arbitration by changing the photo mask used at an exposure process. Therefore, the number of the avidin molecules fixed to the specific part 101 is also controllable to arbitration by changing a photo mask.
[0009] Next, the DNA microarray concerning this invention is explained. The DNA microarray concerning this invention is manufactured by each specific part 101 of the surface-preparation substrate 100 manufactured by the approach of drawing 1 by carrying out the spot of the solution containing the probe DNA from which a base sequence differs respectively. An example of the manufacture approach of the DNA microarray which starts this invention at drawing 3 is shown. 110 are AREIYA for carrying out the spot of the solution 111 containing probe DNA 21 on

the DNA microarray substrate 100 among drawing. DNA (biotin-ized DNA) which carried out the indicator of the biotin beforehand is used for probe DNA 21. AREIYA 110 is supplied to the part 101 of specification [the solution 111 of a constant rate] by dashing the tip of AREIYA 110 which it had come to be able to carry out the constant-rate maintenance of the solution 111 by capillarity, and held the solution 111 against the specific part 101 on the surface treatment substrate 100. Consequently, the probe DNA 21 in a solution 111 combines with each one avidin molecule 22 of every currently fixed to the specific part 101 on the DNA microarray substrate 100 (solid-phase-izing of biotin-ized DNA). Since the number of the avidin molecules 22 currently fixed to each specific part 101 is the same, the number of the probe DNA 21 combined with each specific part 101 is also the same (refer to drawing 4). The number of the avidin molecules fixed to each specific part 101 can be changed into arbitration by changing the photo mask used at an exposure process, and changing the specific configuration and specific dimension of a part 101, as mentioned above. Therefore, it is controllable by modification of the photo mask used at an exposure process to arbitration, and the number of the probe DNA 21 fixed to each specific part 101 can also always fix a fixed number of probe DNA 21 to each specific part 101, even if dispersion is in the probe DNA concentration of the solution 111 which carries out a spot to each specific part 101.

[0010] The example of measurement of the relation between the amount of DNA in the solution which carries out a spot to drawing 5 to the specific part on a surface treatment substrate (concentration), and the amount of DNA fixed by the specific part is shown. This measurement result shows that the amount of probe DNA fixed by the specific part becomes fixed, if the spot of the 3x109 to 5x109 probe DNA is carried out. All the DNA detection spot sections on a DNA microarray are the same configuration and the same area, and since same number [every] probe DNA 21 is moreover being fixed to all the detection spot sections, according to this DNA microarray, quantitive analysis is attained. Since the DNA adsorption site on a DNA microarray is restricted to the specific part 101, i.e., the DNA detection spot section, DNA can prevent that an un-unique target causes adsorption around the DNA detection spot section. Therefore, a S/N ratio can be raised by reduction of the noise at the time of fluorescence detection (unnecessary light). Furthermore, a S/N ratio can be raised more by making it in agreement with the configuration of the pixel of the solid state image sensors (a CCD sensor, CMOS sensor, etc.) which use the configuration of the DNA detection spot section, i.e., the configuration of said specific part, for an image pick-up.

[0011] In addition, this invention is not limited to the gestalt of the above operation. For example, the manufacture approach of the surface treatment substrate concerning this invention is not limited to the gestalt of the above-mentioned operation. That is, by the manufacture approach shown in drawing 1 , the laminating of the photoresist 13 of the aluminum film 12 and a positive type is first carried out to the front face of a substrate 11 one by one. Next, it lets the photo mask which has arranged the specific part 101 regularly pass, and only the specific part of a photoresist is exposed, by dipping in a developer, a photoresist dissolves only the specific part 101 and the aluminum film 12 exposes only the specific part 101. Then, by etching the aluminum film 12 with an acid etching solution, the aluminum film 12 of the specific part 101 is dissolved, and the glass front face of the specific part 101 of a substrate 11 is exposed. It can leave the DNA solid phase-ized film 15 only to the specific part 101 of the front face of a substrate 11 by applying the DNA solid phase-ized film 15 on it, and dissolving the aluminum film 12. As another approach, the DNA solid phase-ized film 15 may be formed in the whole front face of a substrate 11 from the beginning, and only the specific part 101 may adopt the approach of exposing the DNA solid phase-ized film 15. In this case, after carrying out laminating formation of the DNA solid phase-ized film 15 and the aluminum film 12 one by one on the whole front face of a substrate 11, only the specific part 101 can expose the DNA solid phase-ized film 15 by carrying out laminating formation of the photoresist 13 of a positive type on the aluminum film 12, exposing only to the specific part 101 through a photo mask, and performing development and etching like the above. Moreover, a photoresist does not need to be a positive type and it is undoubted that the photoresist of a negative mold is also usable.

[0012] Moreover, although the gestalt of the above-mentioned implementation explained the

DNA microarray, i.e., the biomolecule microarray which fixed DNA as probe biomolecule, the thing using RNA, PNA, protein, etc. as probe biomolecule is also contained in the biomolecule microarray of this invention. Moreover, the substrate used for a surface treatment substrate may not be restricted to a slide glass substrate, and a transparence glass substrate, a silicon substrate, a plastic plate, a golden substrate, a silver substrate, etc. are sufficient as it. Moreover, what is necessary is just to use the matter considered to be the most suitable for quantification of the number of probe biomolecules to fix in consideration of affinity with probe biomolecule to fix not only to what fixed avidin (exposure) but to said specific part, even if attached to a solid phase-ized agent.

[0013]

[Effect of the Invention] As explained above, this invention does the following outstanding effectiveness so. Since surface preparation of the substrate for biomolecule microarrays concerning this invention is carried out so that probe biomolecule may adhere only to the specific part on the front face of a substrate, by carrying out the spot of the solution containing probe biomolecule to a substrate front face, it can prevent that probe biomolecule adheres to the field besides the biomolecule detection spot section, and can obtain the high biomolecule microarray of a S/N ratio. Moreover, the amount of the probe biomolecule fixed in the biomolecule detection spot section is controllable by changing said specific area and specific configuration of a part. The same, then the biomolecule microarray which can make regularity the amount of the probe biomolecule of all the biomolecule detection spot sections, and starts this invention are obtained in all of said specific area and specific configuration of a part. Since the amount of the probe biomolecule fixed by each biomolecule detection spot section is fixed according to the biomolecule microarray concerning this invention, it can be used for the quantitive analysis of target biomolecule. Moreover, since the biomolecule detection spot section is formed by fixing probe biomolecule to a specific part, the nonspecific adsorption of the target biomolecule in the field besides the biomolecule detection spot section can be prevented, and high measurement of a S/N ratio can be performed. According to the manufacture approach concerning this invention, the surface treatment substrate for biomolecule microarrays concerning this invention can be manufactured with high precision by forming only in the specific part on the front face of a substrate the solid phase-ized film which adsorbs probe biomolecule and solid-phase-izes it using high precision processing technology, such as a photolithography.

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TECHNICAL FIELD

[Field of the Invention] The invention in this application uses as a probe biomolecule of the single strand which has a complementary base sequence to the target biomolecule which should be detected, belongs to the biomolecule detection technique detect target biomolecule by detecting the existence of the double strand formed of the hybridization of the probe biomolecule concerned and the sample nucleic acid of the living body origin, and relates to the biomolecule microarray which comes to form the biomolecule detection spot section on the substrate concerned by carrying out the spot of the solution which contains probe biomolecule especially on the surface of a substrate.

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PRIOR ART

[Description of the Prior Art] There is a DNA microarray (called a DNA chip) as a device for detecting the biomolecules (DNA, RNA, etc.) which exist in the sample of the living body origin. According to the DNA microarray, it is possible for biomolecule detection processing or base-sequence-determination processing of hundreds - tens of thousands batches to be put in block, and to perform it in juxtaposition. A DNA microarray comes to arrange the DNA detection point (spot section) of hundreds - a-10,000 number on a several square centimeters - about ten square centimeters glass substrate or a silicon substrate tidily. One kind of nucleic-acid polymer (gene fragment) of a single strand which had a known base sequence beforehand is being fixed to each DNA detection point at a time as a probe (sensor). That is, on a DNA microarray, many kinds of nucleic acid probes have aligned. If the water solution of the sample nucleic acid which carried out labeling (indicator) with the fluorescent material on this DNA microarray is poured, only when the base sequence of the nucleic-acid polymer in a sample nucleic acid is as complementary as FUROBU, both will hybridize, and also even in after washing, only a probe and the target nucleic-acid polymer to which it hybridized remain on a DNA microarray. By detecting the fluorescence which the fluorescent material in the target nucleic-acid polymer which remained on the DNA microarray emits, it can judge whether a target nucleic-acid polymer exists in a sample nucleic acid.

[0003] A DNA microarray can be divided roughly into two kinds, a photolithography mold and a spotting mold, according to a manufacturing method. In the photolithography mold, the manufacture approach which compounds DNA (oligonucleotide) of desired varieties on a substrate (or sheet) by the photolithography used in the manufacture process of a semiconductor integrated circuit is taken, and the DNA microarray which has the DNA detection point of high density is put in practical use (refer to U.S. Pat. No. 5744305 and 5445934 grade). On the other hand, in a spotting mold, after carrying out the spots of the waterdrop containing the probe DNA which prepared beforehand the solid phase-ized agent (the poly lysine or amino silane) on the substrate using the substrate (or sheet) with which the whole surface of slide glass was coated one by one and carrying it, the manufacture approach which forms a DNA detection spot is taken by making it dry (reference, such as U.S. Pat. No. 587522).

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EFFECT OF THE INVENTION

[Effect of the Invention] As explained above, this invention does the following outstanding effectiveness so. Since surface preparation of the substrate for biomolecule microarrays concerning this invention is carried out so that probe biomolecule may adhere only to the specific part on the front face of a substrate, by carrying out the spot of the solution containing probe biomolecule to a substrate front face, it can prevent that probe biomolecule adheres to the field besides the biomolecule detection spot section, and can obtain the high biomolecule microarray of a S/N ratio. Moreover, the amount of the probe biomolecule fixed in the biomolecule detection spot section is controllable by changing said specific area and specific configuration of a part. The same, then the biomolecule microarray which can make regularity the amount of the probe biomolecule of all the biomolecule detection spot sections, and starts this invention are obtained in all of said specific area and specific configuration of a part. Since the amount of the probe biomolecule fixed by each biomolecule detection spot section is fixed according to the biomolecule microarray concerning this invention, it can be used for the quantitive analysis of target biomolecule. Moreover, since the biomolecule detection spot section is formed by fixing probe biomolecule to a specific part, the nonspecific adsorption of the target biomolecule in the field besides the biomolecule detection spot section can be prevented, and high measurement of a S/N ratio can be performed. According to the manufacture approach concerning this invention, the surface treatment substrate for biomolecule microarrays concerning this invention can be manufactured with high precision by forming only in the specific part on the front face of a substrate the solid phase-ized film which adsorbs probe biomolecule and solid-phase-izes it using high precision processing technology, such as a photolithography.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] There is a difference among the following properties in two kinds of DNA microarrays mentioned above. Since the DNA microarray of a photolithography mold can make the DNA detection point fine and can grow DNA to homogeneity, it is excellent in the point that high sensitometry and its repeatability can be guaranteed, and the point which can be used for SNP (a little salt radical polymorphism) analysis. However, one mask is required in order to compound one base, and since there are four kinds of bases with A, T, G, and C, at least four masks are needed. For example, 80 masks are required to compound the probe of the die length of 20 bases. The mask is as expensive as hundreds of thousands of yen per sheet, and in order to make a DNA microarray, it requires tens of millions of yen costs. For this reason, the present condition is used only by some research facilities. [0005] In order to use for the DNA microarray of a spotting mold the approach of carrying the waterdrop containing probe DNA on a substrate, and drying it, the consistency and uniformity of DNA which are fixed on a substrate are not guaranteed. That is, since the dimension and configuration of the DNA detection spot section become an ununiformity, dispersion arises in the amount of DNA currently fixed to each DNA detection spot section. For this reason, even if the DNA microarray of a spotting mold was applicable to qualitative analysis, it was not fit for quantitive analysis. That is, even if the existence of the DNA detection spot section which the hybridization with target biomolecule produced was detectable, it was not able to measure the amount of the target biomolecule which carried out hybridization in each DNA detection spot section. Moreover, by existence of the solid phase-ized agent adhering to the perimeter of the DNA detection spot section, Target DNA adsorbed on the substrate nonspecific, the rise of a noise was caused, and the S/N ratio was reduced. It is originated under such a situation and the invention in this application has the purpose in being able to use it for quantitive analysis and offering the biomolecule microarray of the high spotting mold of a S/N ratio.

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MEANS

[Means for Solving the Problem] In order to attain the above-mentioned purpose, the following means are adopted in the invention in this application. The substrate for biomolecule microarrays concerning this invention is characterized by preparing regularly the detailed probe biomolecule acceptance solid phase section of the plurality on the front face of a substrate which migrates to the whole surface mostly and may receive said probe biomolecule quantitatively respectively. In the substrate for biomolecule microarrays of this invention, said probe biomolecule acceptance solid phase section consists of a solid phase-ized agent of the avidin, streptoavidin, a biotin, the amino group, a carbonyl group, a hydroxyl group, a SUKUSHI need radical, a MAREIDO radical, or the thiol groups. Moreover, said substrates are a glass substrate, a silicon substrate, a plastic plate, a golden substrate, or the silver substrates. Moreover, an avidin molecule combines said probe biomolecule acceptance solid phase section with the end of the biotin molecule combined with the substrate front face at a monolayer. Moreover, as for spacing of 50-200 microns and said specific parts, it is [the path of said specific part] desirable that it is 100-500 microns. Here, as for the case of a diameter and a square, the path of said specific part means the die length of a piece, when the configuration of the specific part concerned is circular. Moreover, it is desirable for the configuration of said specific part to carry out abbreviation coincidence with the configuration of the pixel of the solid state image sensor used for the image pick-up of the biomolecule detection spot section of said biomolecule microarray. The biomolecule microarray concerning this invention is characterized by combining probe biomolecule with said probe biomolecule acceptance solid phase section of a substrate according to claim 1 to 4. In the biomolecule microarray of this invention, said probe biomolecule is DNA, RNA, PNA, or protein. Moreover, said probe biomolecule is the biomolecule which carried out the indicator of the biotin, and is combined with said probe biomolecule acceptance solid phase section by biotin-avidin association. The manufacture approach concerning this invention is an approach of manufacturing said substrate for biomolecule microarrays, and is characterized by including the process in which only a specific part prepares said probe biomolecule acceptance solid phase section using a photolithography technique and an etching technique.
[0007]
[Embodiment of the Invention] Hereafter, the gestalt of operation of this invention is explained to a detail with reference to a drawing. First, the substrate for DNA microarrays concerning this invention (it is only hereafter described as a surface treatment substrate.) is explained. Drawing 1 is the production process Fig. showing an example of the manufacture approach of the surface treatment substrate concerning this invention. Among drawing, 100 are a surface-preparation substrate concerning this invention, surface preparation is carried out and this surface-preparation substrate 100 becomes so that probe DNA may adhere only to the specific part 101. The production process of the surface treatment substrate 100 is as follows.
(1) Substrate washing process : wash the slide glass substrate 11 and remove an impurity.
(2) Aluminum film vacuum evaporationo process : vapor-deposit the aluminum film 12 on the front face of the slide glass substrate 11 (coating).
(3) The spreading process of a photoresist : apply the photoresist of a negative mold to the front face of the aluminum film 12 (coating).

(4) Exposure process : irradiate light (hnu) only to the specific part 101 on the substrate of (3) through a photo mask 14.
(5) Develop the photoresist 13 on the substrate of development process: (4). The photoresist 13 of the specific part 101 is removed in this phase.
(6) Etch the aluminum film on the substrate of etching process: (5). In this phase, the aluminum film 12 of the specific part 101 is removed.
(7) Dissolve with an acetone and remove the photoresist 13 on the substrate of resist removal process: (6). In this phase, the front face of the slide glass substrate 11 exposes only the specific part 101.
(8) On the substrate of DNA solid phase-ized film formation process: (7), apply the solid phase-ized agent which adsorbs probe DNA and solid-phase-izes it, and form the DNA solid phase-ized film 15. Specifically, this process consists of an amino-group installation process on the front face of a substrate by amino silanizing, and a biotin installation process to the amino group on the front face of a substrate by the biotin SUKUSHI need.
(9) Make it dissolve by the acid, alkali, or the chelating agent, and remove the aluminum film 12 on the substrate of DNA attachment site formation process: (8). The DNA solid phase-ized film 15 is formed only in the specific part 101 of the front face of the slide glass substrate 11 in this phase.
(10) Introduce an avidin solution on the substrate of avidin joint process: (9), and make a monolayer combine an avidin molecule with the end of the biotin molecule of the DNA solid phase-ized film 15 formed in the specific part 101.
By passing through the process of the above (1) - (10), the DNA microarray substrate 100 with which avidin was fixed only to the specific part 101 of the front face of the slide glass substrate 11 by the monolayer is obtained. Spacing of 200 microns or less and part 101 specific comrades of the diameter of the specific part 101 is 400 microns or less.
[0008] The process which an avidin molecule combines with a monolayer is shown in the end of the biotin molecule of each DNA solid phase-ized film 15 at drawing 2 . Since all of a specific area and the specific configuration of a part 101 which are formed in the front face of each substrate are homogeneity, its number of the biotin molecules 23 fixed to each specific part 101 is also almost uniform. Therefore, the number of the avidin molecules combined with each specific part 101 becomes equal. That is, even if some dispersion is in the number of the biotins fixed to each specific part 101, the number of the avidin molecules with which the direction of an avidin molecule is fixed to each specific part 101 since it is larger than a biotin molecule for whether your being Haruka becomes fixed. Spacing of the configuration of the specific part 101, a dimension, and part 101 specific comrades can be changed into arbitration by changing the photo mask used at an exposure process. Therefore, the number of the avidin molecules fixed to the specific part 101 is also controllable to arbitration by changing a photo mask.
[0009] Next, the DNA microarray concerning this invention is explained. The DNA microarray concerning this invention is manufactured by each specific part 101 of the surface-preparation substrate 100 manufactured by the approach of drawing 1 by carrying out the spot of the solution containing the probe DNA from which a base sequence differs respectively. An example of the manufacture approach of the DNA microarray which starts this invention at drawing 3 is shown. 110 are AREIYA for carrying out the spot of the solution 111 containing probe DNA 21 on the DNA microarray substrate 100 among drawing. DNA (biotin-ized DNA) which carried out the indicator of the biotin beforehand is used for probe DNA 21. AREIYA 110 is supplied to the part 101 of specification [the solution 111 of a constant rate] by dashing the tip of AREIYA 110 which it had come to be able to carry out the constant-rate maintenance of the solution 111 by capillarity, and held the solution 111 against the specific part 101 on the surface treatment substrate 100. Consequently, the probe DNA 21 in a solution 111 combines with each one avidin molecule 22 of every currently fixed to the specific part 101 on the DNA microarray substrate 100 (solid-phase-izing of biotin-ized DNA). Since the number of the avidin molecules 22 currently fixed to each specific part 101 is the same, the number of the probe DNA 21 combined with each specific part 101 is also the same (refer to drawing 4). The number of the avidin molecules fixed to each specific part 101 can be changed into arbitration by changing the photo

mask used at an exposure process, and changing the specific configuration and specific dimension of a part 101, as mentioned above. Therefore, it is controllable by modification of the photo mask used at an exposure process to arbitration, and the number of the probe DNA 21 fixed to each specific part 101 can also always fix a fixed number of probe DNA 21 to each specific part 101, even if dispersion is in the probe DNA concentration of the solution 111 which carries out a spot to each specific part 101.

[0010] The example of measurement of the relation between the amount of DNA in the solution which carries out a spot to drawing 5 to the specific part on a surface treatment substrate (concentration), and the amount of DNA fixed by the specific part is shown. This measurement result shows that the amount of probe DNA fixed by the specific part becomes fixed, if the spot of the 3x109 to 5x109 probe DNA is carried out. All the DNA detection spot sections on a DNA microarray are the same configuration and the same area, and since same number [every] probe DNA 21 is moreover being fixed to all the detection spot sections, according to this DNA microarray, quantitive analysis is attained. Since the DNA adsorption site on a DNA microarray is restricted to the specific part 101, i.e., the DNA detection spot section, DNA can prevent that an un-unique target causes adsorption around the DNA detection spot section. Therefore, a S/N ratio can be raised by reduction of the noise at the time of fluorescence detection (unnecessary light). Furthermore, a S/N ratio can be raised more by making it in agreement with the configuration of the pixel of the solid state image sensors (a CCD sensor, CMOS sensor, etc.) which use the configuration of the DNA detection spot section, i.e., the configuration of said specific part, for an image pick-up.

[0011] In addition, this invention is not limited to the gestalt of the above operation. For example, the manufacture approach of the surface treatment substrate concerning this invention is not limited to the gestalt of the above-mentioned operation. That is, by the manufacture approach shown in drawing 1 , the laminating of the photoresist 13 of the aluminum film 12 and a positive type is first carried out to the front face of a substrate 11 one by one. Next, it lets the photo mask which has arranged the specific part 101 regularly pass, and only the specific part of a photoresist is exposed, by dipping in a developer, a photoresist dissolves only the specific part 101 and the aluminum film 12 exposes only the specific part 101. Then, by etching the aluminum film 12 with an acid etching solution, the aluminum film 12 of the specific part 101 is dissolved, and the glass front face of the specific part 101 of a substrate 11 is exposed. It can leave the DNA solid phase-ized film 15 only to the specific part 101 of the front face of a substrate 11 by applying the DNA solid phase-ized film 15 on it, and dissolving the aluminum film 12. As another approach, the DNA solid phase-ized film 15 may be formed in the whole front face of a substrate 11 from the beginning, and only the specific part 101 may adopt the approach of exposing the DNA solid phase-ized film 15. In this case, after carrying out laminating formation of the DNA solid phase-ized film 15 and the aluminum film 12 one by one on the whole front face of a substrate 11, only the specific part 101 can expose the DNA solid phase-ized film 15 by carrying out laminating formation of the photoresist 13 of a positive type on the aluminum film 12, exposing only to the specific part 101 through a photo mask, and performing development and etching like the above. Moreover, a photoresist does not need to be a positive type and it is undoubted that the photoresist of a negative mold is also usable.

[0012] Moreover, although the gestalt of the above-mentioned implementation explained the DNA microarray, i.e., the biomolecule microarray which fixed DNA as probe biomolecule, the thing using RNA, PNA, protein, etc. as probe biomolecule is also contained in the biomolecule microarray of this invention. Moreover, the substrate used for a surface treatment substrate may not be restricted to a slide glass substrate, and a transparence glass substrate, a silicon substrate, a plastic plate, a golden substrate, a silver substrate, etc. are sufficient as it. Moreover, what is necessary is just to use the matter considered to be the most suitable for quantification of the number of probe biomolecules to fix in consideration of affinity with probe biomolecule to fix not only to what fixed avidin (exposure) but to said specific part, even if attached to a solid phase-ized agent.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the production process Fig. showing an example of the manufacture approach of the surface treatment substrate concerning this invention.
[Drawing 2] It is an explanatory view about the process which an avidin molecule combines with the end of the biotin molecule of each DNA solid phase-ized film.
[Drawing 3] It is the explanatory view showing an example of the manufacture approach of the DNA microarray concerning this invention.
[Drawing 4] It is an explanatory view about the process which probe DNA (biotin-ized DNA) combines with the avidin molecule currently fixed to the specific part.
[Drawing 5] It is drawing showing the measurement result of the relation between the amount of DNA in the solution which carries out a spot to the specific part on a surface treatment substrate (concentration), and the amount of DNA fixed by the specific part.
[Description of Notations]
11: Slide glass substrate
12: Aluminum film
13: Photoresist
14: Photo mask
15: Solid phase-ized film
21: Probe DNA
22: Avidin molecule
23: Biotin molecule
100: DNA microarray surface treatment substrate
101: A specific part
110: AREIYA
111: Solution

[Translation done.]

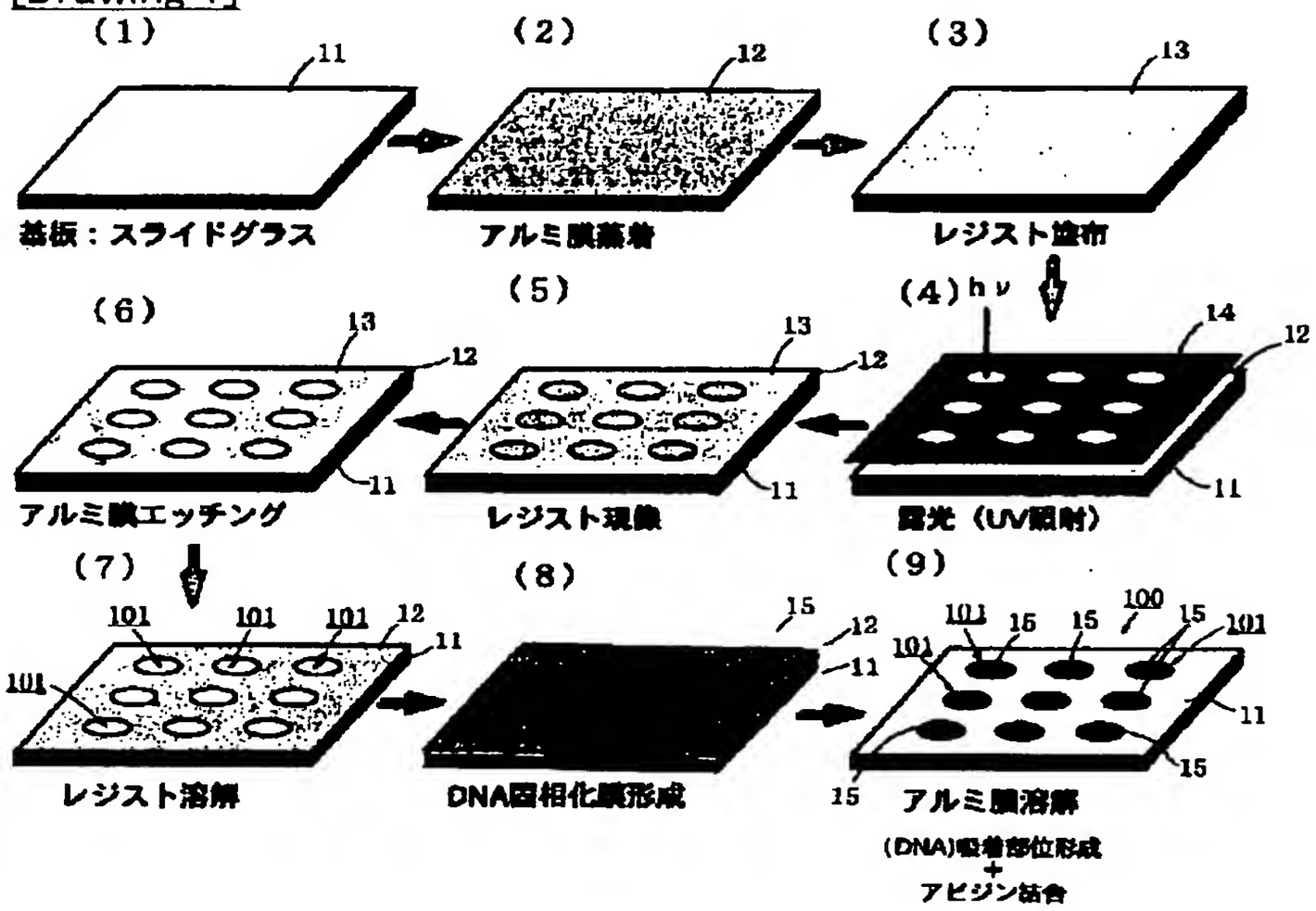
* NOTICES *

JPO and NCIPI are not responsible for any
damages caused by the use of this translation.

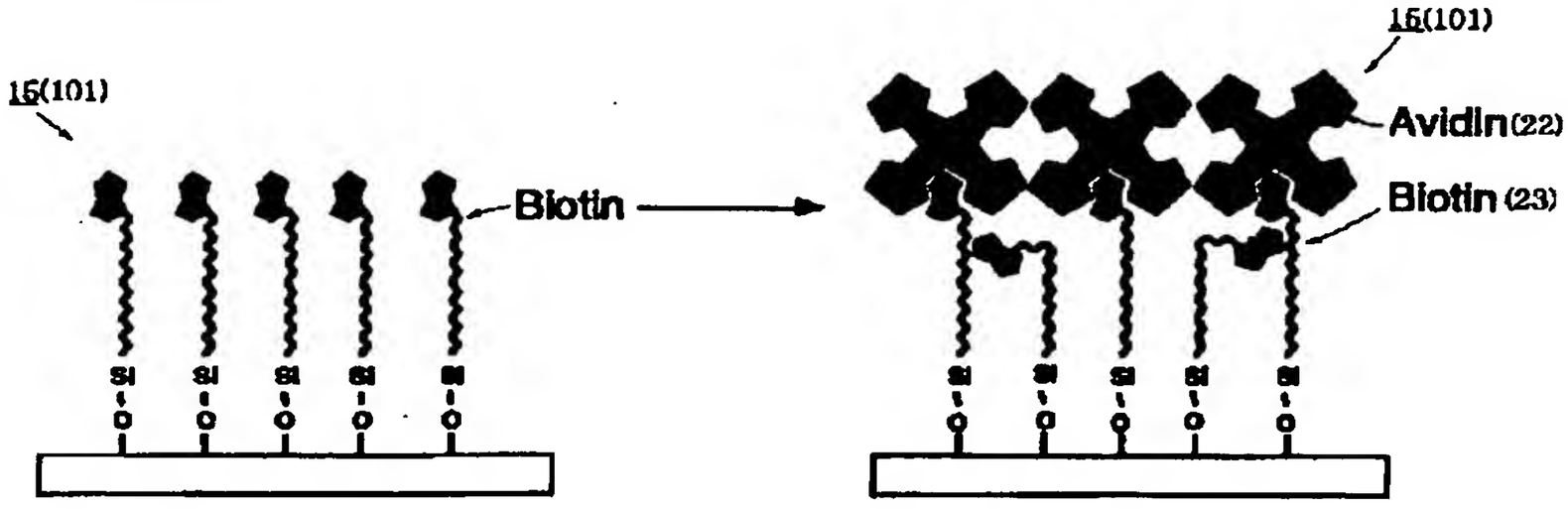
1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

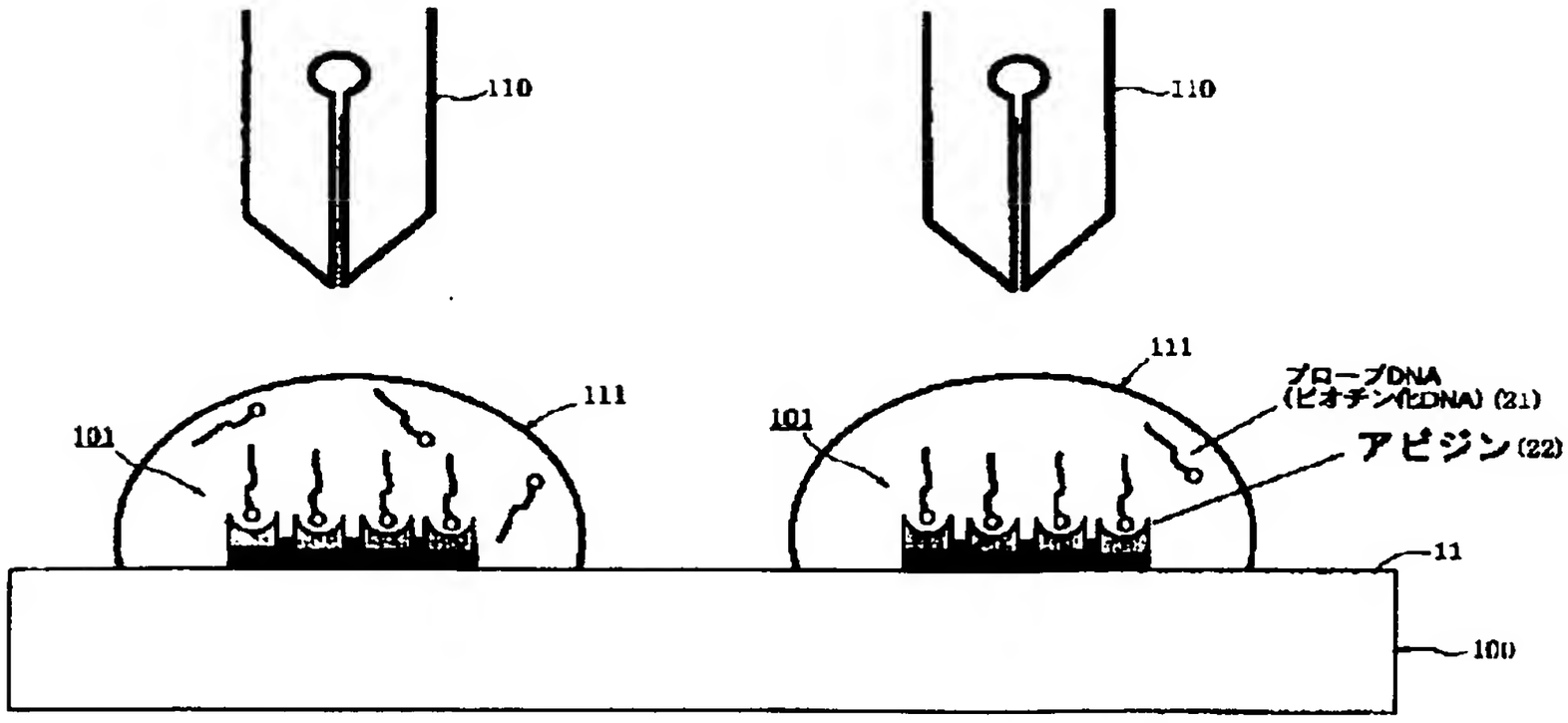
[Drawing 1]



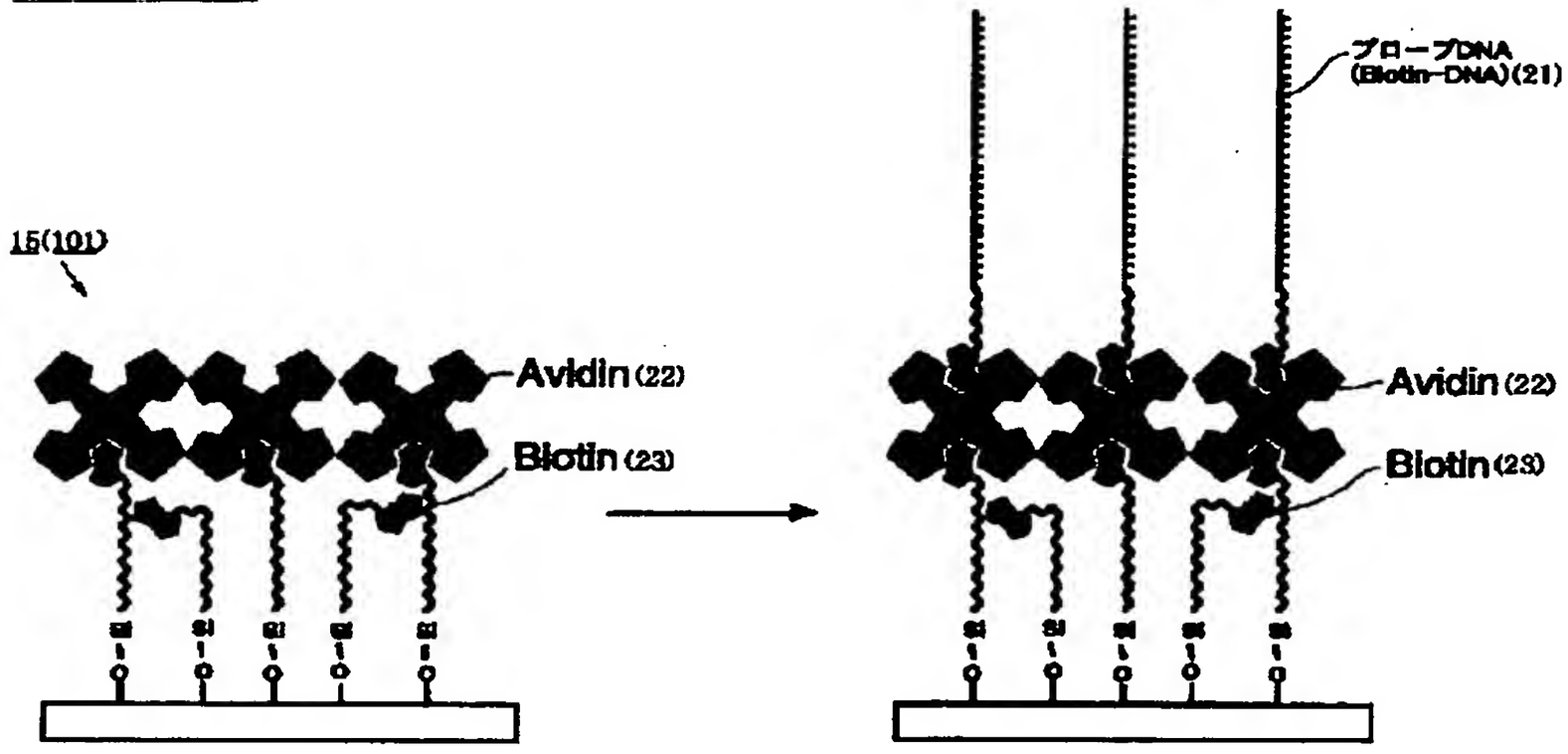
[Drawing 2]



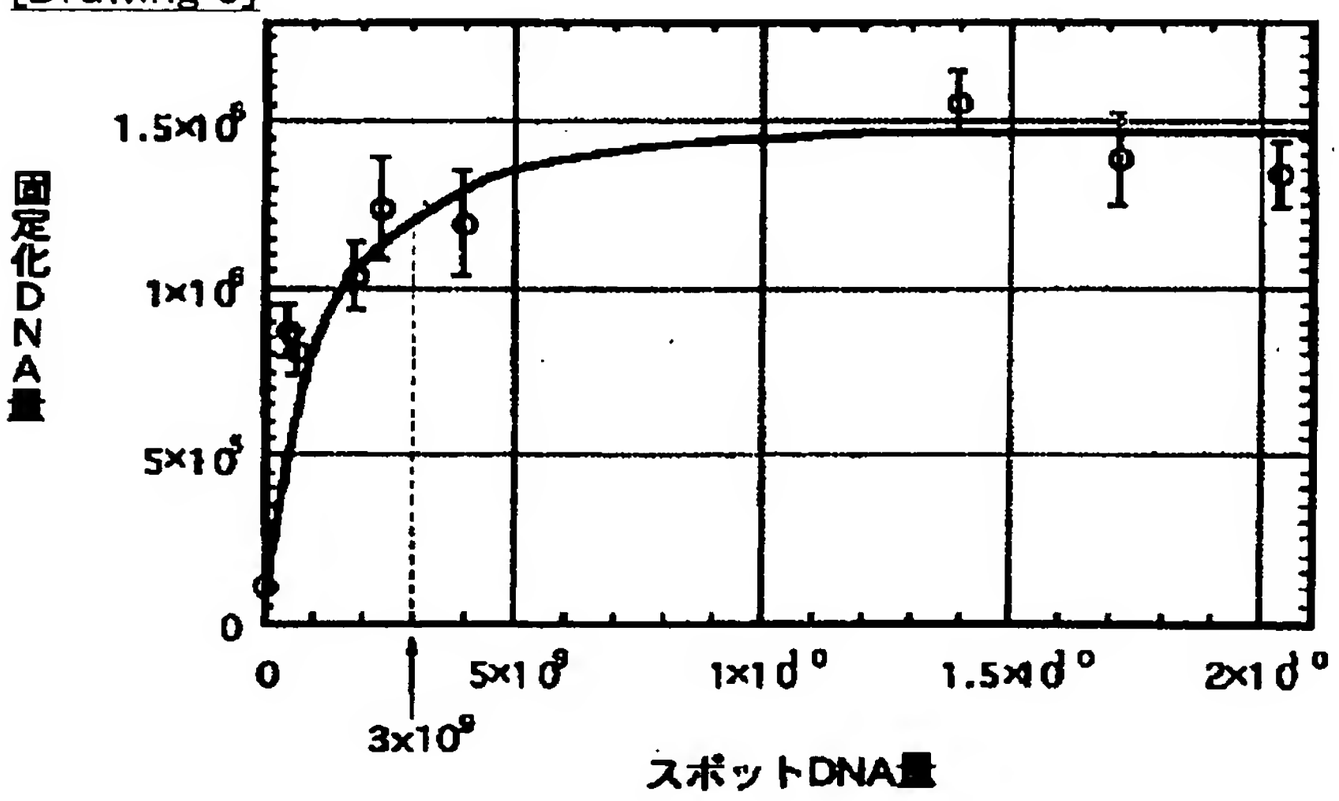
[Drawing 3]



[Drawing 4]



[Drawing 5]



[Translation done.]